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Fluorescent primary sensor for zinc and resultant complex as secondary sensor towards phosphorylated biomolecules: INHIBIT logic gate

Kamalpreet Kaur [a], Vimal K. Bhardwaj [a], Navneet Kaur [b], Narinder Singh [a,*]

[a] Department of Chemistry, Indian Institute of Technology Ropar, Rupnagar, Punjab 140001, India
[b] Centre for Nanoscience & Nanotechnology, Panjab University, Chandigarh, Punjab 160014, India

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ABSTRACT

Imine linked "on–off" multi-responsive and selective chemosensor has been synthesized and evaluated for cation recognition properties. The receptor shows high sensitivity and selectivity for Zn^{2+} through changes in fluorescence intensity based on ESIPT and charge transfer (CT) mechanism. The Zn^{2+} complex of the receptor can be used for phosphate quantification and for recognition of phosphorylated biomolecules through cation displacement approach.

1. Introduction

In recent years, the use of organic compounds for chemosensor development has made remarkable progress [1]. These chemosensors relay through change in magnetic, electronic or optical properties when it binds to any specific analyte. The fluorescent chemosensors have attracted considerable attention due to high sensitivity [2], convenient use and real application in biological systems [3]. In order to achieve practical applications, the future of chemosensors is dependent upon various types of fluorescence mechanisms [4–6]. Recently, the title of "Organic compounds as sensor for metal ion and resultant metal complex as sensor for anion" is gaining popularity. The system works through cation displacement assay [7]: the receptor presents a unique cavity and on binding with the metal ion shows a significant change in the fluorescence profile of the receptor. The resultant metal ion complex acts as an ideal candidate for the recognition of anions through the liberation of the sensor from the metal complex due to the strong affinity of the anion towards the metal ion; consequently bringing a considerable change in the fluorescence profile of the receptor–metal complex. The recognition of anions with metal complexes [8] has advantage as it validates anion detection even in semi-aqueous medium. In addition to the analytical applications, the concept has direct implementation in the development of molecular logic gates [9,10]. The increasing role of such systems in supramolecular devices, as well as constant need to pursue superior technologies, will raise a wide interest of cation displacement assay.

2. Experimental

2.1. Materials and methods

Chemicals were purchased from Sigma Aldrich and were used without further purification. The NMR spectra were recorded on a Avance-II (Bruker) instrument, which operated at 400 MHz for ^1H NMR and ^{13}C NMR in DMSO-d_6. IR spectra were recorded on a Bruker Tensor 27 spectrometer for the compounds in the solid state as KBr discs. The absorption spectra were recorded on a Specord 250 Plus Analytikjena spectrometer spectrophotometer. The fluorescence measurements were performed on a Perkin Elmer L55 Fluorescence spectrophotometer. The mass spectrum of **1**·Zn^{2+} complex was recorded on Waters Micromass Q–T of Micro Instrument.

2.2. Synthesis of compound **1**

This compound was prepared by stirring 4,4′-diaminodiphenylmethane (198 mg, 1.0 mmol) along with 4-(diethylamino)-2-hydroxybenzaldehyde (193 mg, 2.5 mmol) in methanol. The reaction mixture was stirred for 12 h at room temperature. The

* Corresponding author. Tel.: +91 1881242176; fax: +91 1881223395.
E-mail address: nsingh@iitrpr.ac.in (N. Singh).

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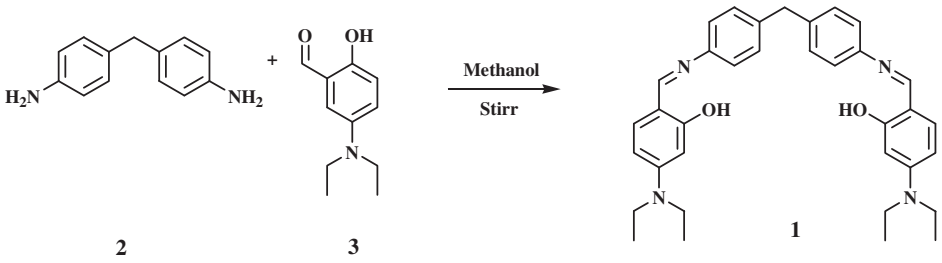

Scheme 1.

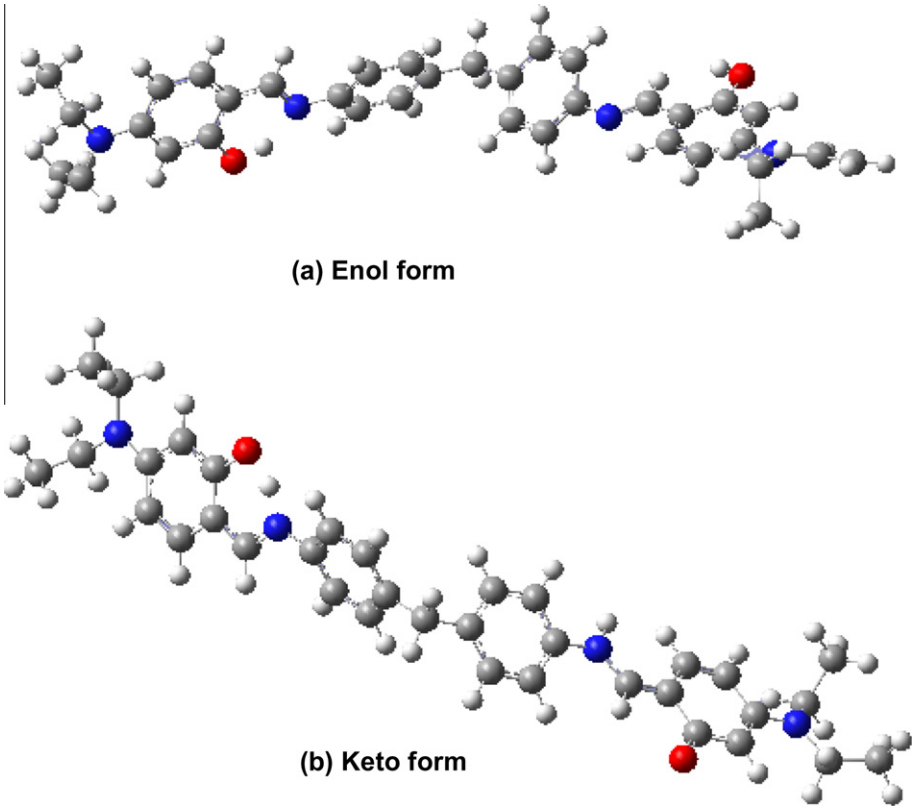

Fig. 1. The optimized structure of receptor **1**: (a) enol form and (b) keto form, calculated at the B3LYP/6-31G* level. The red, blue and gray spheres refer to O, N and C atoms respectively.

yellow colored precipitates were formed during the course of reaction and these precipitates were washed with MeOH. The solid was recrystallized from MeOH, giving a solid with 90% yield and was characterized with IR and NMR spectroscopy. IR (KBr) 3550, 1688, 1101 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 1.13(t, 12H, $-CH_3$), 3.38(q, 8H, $-N-CH_2-$), 3.95(s, 2H, $-CH_2$), 6.06(d, 2H, Ar), 6.31(d, 2H, Ar), 7.20(d, 2H, Ar), 7.28(d, 2H, Ar), 7.31(s, 1H, Ar), 8.66 (s, 2H, –CH=N–), 13.65 (s, 2H, –OH) ^{13}C (100 MHz, DMSO-d_6) δ 12.50, 43.71, 43.88, 96.81, 103.79, 107.20, 108.11, 120.72, 129.62, 134.10, 138.10, 160.50, 161.42, 163.46.

2.3. Cation recognition properties of *1*

The cation recognition studies were performed at 25 ± 1 °C and before recording any spectrum a sufficient time was give to ensure the uniformity of the solution. The cation binding ability of **1** (10 μM) was determined by preparing standard solutions of **1** (10 μM) along with fixed amounts of particular metal nitrate salt (100 μM) in HEPES buffered DMF/H_2O (7:3, v/v). The cation recognition behavior of **1** (10 μM) was evaluated from the changes in fluorescence spectrum of sensor upon addition of that metal salt (100 μM). The fluorescence spectrum of **1** was recorded with excitation wavelengths of 400 nm. For titrations, volumetric flasks were taken each containing standard solution of sensor **1** (10 μM) along with varied amounts of a zinc nitrate salt (0–100 μM) in HEPES buffered DMF/H_2O (7:3, v/v). In order to determine the stoichiometry of the complex formed from receptor **1** and Zn^{2+}, solutions of **1** and Zn^{2+} were prepared as1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. These solutions were kept for 1 h, and were shaken occasionally. The plot of [HG] versus [H]/([H] + [G]) was used to determine the stoichiometry of the complex formed. The fluorescence intensity at 462 nm was used for calculations. The concentration of [HG] was calculated by the equation $[HG] = \Delta I/I_o x\ [H]$. To evaluate any possible interference due to different cations for the estimation of Zn^{2+}, solutions were prepared containing **1** (10 μM) and Zn^{2+} (50 μM) along with both without and with other interfering metal ions (50 μM) in DMF/H_2O (7:3, v/v) HEPES buffered solution (pH = 7.0 ± 0.1). The fluorescence intensity of each solution was recorded at 462 nm.

2.4. Synthesis of **1**·Zn^{2+} complex

Anaqueous solution of zinc nitrate (0.2975 g, 1 mmol) was added to a solution of **1** (0.548 g, 1 mmol) in THF. The solution was refluxed for three hours. On completion of the reaction, the solution was filtered and the solvent evaporated, a yellowish-brown compound was obtained, washed with methanol and dried under vacuum. The dark-brown colored powder was obtained on recrystallization from methanol. Yield 67%. mp = 205 °C. Selected IR (KBr, cm^{-1}): 1709 (s) $\nu_{C=N}$. UV–Vis absorbance:λ_{max}. (nm), $\varepsilon(M^{-1}\,cm^{-1})$ in DMF/H_2O (7:3, v/v)400 (38432). ESI-MS (*m*/*z*): 372.9 $[M+H]^+$, where M = [**1** + Zn^{2+} + NO_3^- + THF].

2.5. Anion recognition properties of **1**·Zn^{2+}

The studies were performed similar to those of cation recognition properties **1**, except that tetrabutyl ammonium salts of anions were used and detailed concentrations are mentioned in the text of manuscript.

3. Result and discussion

3.1. Synthesis of receptor

Receptor **1** was synthesized by stirring 4,4′-diaminodiphenylmethane (198 mg, 1.0 mmol) along with 4-(diethylamino)-2-hydroxybenzaldehyde (193 mg, 2.5 mmol) in methanol as shown in Scheme 1 and was characterized with IR and NMR spectroscopy. The IR spectrum of **1** shows a band at 1688 cm^{-1} corresponding to $\nu_{C=N}$ stretching (Fig. S1), ^1H NMR spectrum of **1** is showing signals at δ 8.66 (s, 2H, –CH=N–), 13.65 (s, 2H, –OH) and ^{13}C NMR spectrum of **1** is showing signals at δ 161.42 and 163.46.

3.2. Photophysical properties of receptor **1** and molecular model calculations

The photo-physical properties of **1** were evaluated in HEPES buffered DMF/H_2O (7:3, v/v) solvent system using UV–Vis absorption and fluorescence spectra. The UV–Vis absorption spectrum of **1** (10 μM) showed a band at 400 nm (Fig. S2) in HEPES buffered DMF/H_2O (7:3, v/v) solvent system. The band is because of intramolecular charge transfer with imine linkage. The fluorescence spectra of receptor **1** upon excitation at λ_{max} = 400 nm (at room temperature) showed emission bands at λ_{max} = 457 and 509 nm in HEPES buffered DMF/H_2O (7:3 v/v) solvent system. The dual channel emission is explained on the basis of excited state intramolecular proton transfer (ESIPT) process involving keto and enol tautomers. The modified Jablonski diagram exhibiting the ESIPT phenomenon is shown in Fig. S3. The ESIPT process involves the quick transfer of a hydroxyl proton to imine nitrogen through a six- or five membered ring hydrogen-bonding configuration. The enol So ground state is photo excited and it transforms into the enol S1 excited state which then changes into the more stable keto S1[11], photo excited tautomer, which in turns decays to the ground state in a radiative way. The longer wavelength band noticed at 509 nm is due to keto form and the emission band found at 457 nm is assigned as fluorescence emission from the excited enol state. The density functional theory using Becke's three parameterized Lee–Yang–Parr (B3LYP) exchange functional with 6-31G* basis sets, on Gaussian-09 programs [12] revealed the optimized structures (Fig. 1). The optimized structures of enol form and the keto form (Fig. 1A and B) showed that the structures are unsymmetrical. One part of the structures has the geometry suitable for the proton transfer through ESIPT, the second part of the same molecule has the geometrical restriction, where –OH group lies far apart from the nitrogen of –CH=N to exhibit ESIPT. The thermodynamic criteria to show ESIPT process is that the energy of the ground state keto form should be lower than that of the enol state i.e. [$E_{keto}(S_1) < E_{Enol}(S_1)$]. The molecular orbital diagrams show that energy gap between HOMO–LUMO of the keto form of **1** is lesser (E = 0.68409 eV) than the energy gap between HOMO–LUMO of the enol form (1.57174 eV) (Fig. S4).

3.3. Metal recognition properties

In order to evaluate the cation recognition behavior of **1**, the changes in fluorescence spectrum of receptor **1** were recorded upon addition of a particular metal salt (Fig. 2A). Upon addition of 100 μM solution of Zn^{2+} to the 10 μM solution of **1**, a new band was emerged at λ_{max} = 462 nm. Under the same conditions (as used for Zn^{2+}), the fluorescence response of **1** was checked for other metal ions such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Al^{3+}, Cr^{3+}, Fe^{3+}, Mn^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Ag^+, Hg^{2+} and Pb^{2+} (Fig. 2 A). The addition of Ba^{2+}, Cd^{2+} and Pb^{2+} resulted in precipitation; however, no significant fluorescence change of **1** was detected in the presence of other tested metal ions. The observed band for Zn^{2+} complex of **1** is explained as follows: receptor **1** is exhibiting keto–enol tautomerism with equilibrium in the two tautomeric forms as also projected from density functional theory (DFT) results by having approximately equal energies for enol (−1726.75 a.u.) and keto (−1726.81 a.u.). Ideally, dual channel emission is exhibited by the receptor if keto and enol forms exist in equilibrium with each other. Upon addition of Zn^{2+}, it seems that enol form is binding with Zn^{2+}, thus the enol form is eliminated from the equilibrium; consequently shifting the equilibrium towards enol form and fluorescent enhancement is observed. Moreover the enhancement is not observed exactly at 457 nm. The binding of Zn^{2+} close to fluo-

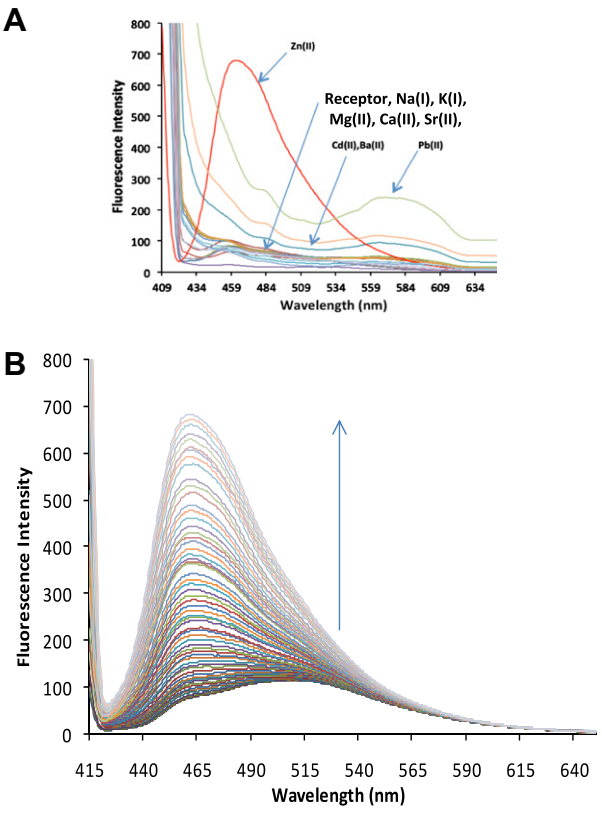

Fig. 2. (A) Fluorescence spectra of **1** (10 μM) upon addition of 100 μM of different metal nitrate salts in HEPES buffered DMF/H_2O (7:3, v/v) solvent system; (B) Fluorescence emission spectra of **1** (10 μM) upon successive addition of Zn^{2+} (0–100 μM) in HEPES buffered DMF/H_2O (7:3, v/v); excited at 400 nm.

rophore may have influenced the ring currents and thus resulted in the modulation of the charge transfer (CT) band. Thus the observed band is the consequence of two phenomena i.e. ESIPT and modulation of CT band [13].

In order to investigate the properties of **1** as a sensor forZn^{2+}, a fluorescence titration was carried out by adding aliquots of Zn^{2+} (0–100 μM) to the solution of **1** in HEPES buffered DMF/H_2O (7:3, v/v) (Figure 2B). The addition of incremental amounts of Zn^{2+} (0–100 μM) to the solution of compound **1** resulted in emergence of anew band and seven fold enhancement of fluorescence intensity at 462 nm, which is exactly same as observed in metal binding test shown in Fig. 2A. The detection limit of **1** [14] as a fluorescent sensor for the analysis of Zn^{2+} was determined from a plot of normalized fluorescence intensity as a function of the concentration of the added metal ions and it was found that **1** has a detection limit of 10 μM for Zn^{2+}. The association constant K_a of **1** for zinc was calculated on the basis of the Benesi–Hildebrand plot [15] (Fig. S5) and it was found to be 2.2×10^3 M^{-1}. The observed selectivity of Zn^{2+} can be explained in terms of hard-hard interactions between Zn^{2+} and donor sites, as compare to soft-hard interactions between other metal ions (with d^{10} configuration) and donor sites. On addition of Zn^{2+} to the solution of receptor, the coordination of Zn^{2+} occurs through hard donors, imine nitrogens and is further facilitated by the binding from a polar −OH group. It has been seen that the availability of a pseudo-cavity provided by imine N of the ligands, which is supported by chelation through –OH group and size difference of zinc as compare to other metal ions has an important role in cation binding and selectivity. Hence although both Ag^+/Hg^{2+} and Zn^{2+} ions have similar electronic configurations, however change of florescence intensity of receptor was seen only in presence of Zn^{2+} ion. The stoichiometry of the complex formed was determined by Job's plot [16] at 462 nm (Fig. S6) and it was found to be 1:1. In order to evaluate the reaction time for zinc sensing, the time-dependence of fluorescence intensity of receptor **1** was checked and results were compared to other metal ions (Fig. S7). Receptor **1** reached equilibrium immediately upon mixing with the solution of Zn^{2+}.

3.4. Competitive metal binding test

To check the practical applicability of **1** as selective fluorescence sensor for Zn^{2+}; a competitive experiment was performed for the estimation of Zn^{2+} (5 equiv.) in the presence of any of Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Al^{3+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Cu^{2+}, Ag^+, Hg^{2+} or Pb^{2+}(5 equiv.). No significant difference in the intensity was observed by comparing the intensity with and without other metal ions (Fig. S8). The distinctive binding of Zn^{2+} suggests an interesting panorama to develop receptor **1** as a sensor for Zn^{2+} even in the presence of other metal ions.

3.5. Anion recognition studies of zinc complex of receptor *1*

The zinc complex of receptor **1** was synthesized by refluxing the aqueous solution of zinc nitrate with a solution of **1** in THF. The **1**·Zn^{2+} complex has been characterized by ESI Mass, IR and compared through fluorescence spectroscopy. The mass spectrum showed *m*/*z* at 372.9, which correspond to $[M+1]^+$ where M is [**1** + Zn^{2+} + THF + NO_3^-] (Fig. S9). The IR spectra of **1**·Zn^{2+} complex indicates that the $\nu_{C=N}$ stretching band of **1** on complexation with Zn^{2+} was shifted to a higher frequency by 21 cm^{-1}confirming that

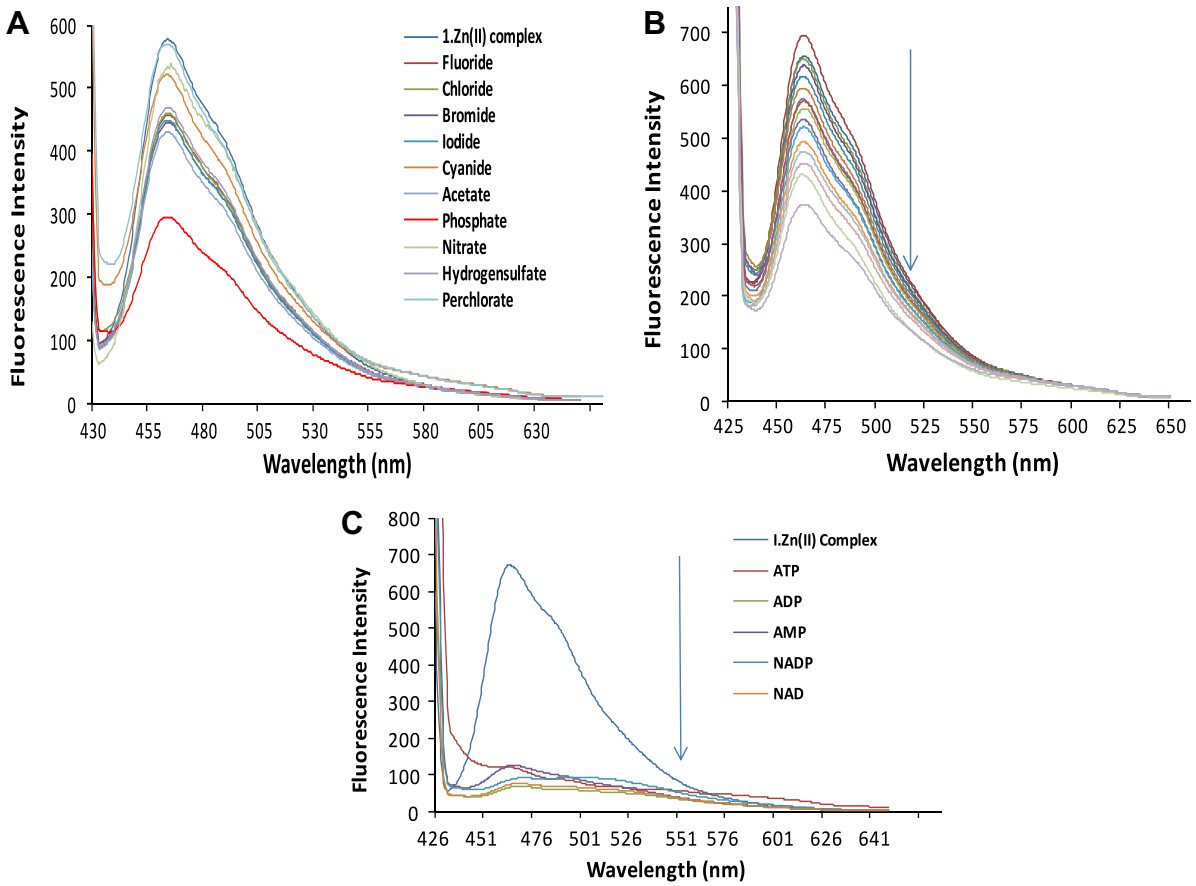

Fig. 3. (A) Changes in fluorescence intensity of **1**·Zn^{2+} (10 μM) upon addition of 20 μM of tetrabutyl ammonium salts of different anions in DMF/H_2O (7:3, v/v) solvent system; (B) Fluorescence emission spectra of **1**·Zn^{2+}(10 μM) upon addition of PO_4^{3-} (0–20 μM) in DMF/H_2O (7:3, v/v); (C) Changes in fluorescence intensity of **1**·Zn^{2+} (10 μM) upon addition of 50 μM of phosphorylated species in DMF/H_2O (7:3, v/v) solvent system.

imine linkages of receptor **1** are coordinating with Zn^{2+} (Fig. S1). The effect of anions on the fluorescence profile of **1**·Zn^{2+} was examined in HEPES buffered DMF/H_2O (7:3, v/v) solvent system. The fluorescence profile of **1**·Zn^{2+} complex showed no considerable change in the presence of any of F^-, Cl^-, Br^-, I^-, CN^-, ClO_4^-, NO_3^-, CH_3COO^- and HSO_4^-. However, addition of phosphate to the **1**·Zn^{2+} complex leads to quench the band at 462 nm. Out of the tested anions, maximum quenching was observed in case of phosphate (Fig. 3A). The selective binding of phosphate ion to **1**·Zn^{2+} complex can be explained due to the total anionic charge density of all the O–P oxygen atoms that participate in the complex formation between phosphate ion and zinc ion. In contrast to this, all other anions have lesser charge density in comparison to phosphate ion. Hence a significant quenching is observed upon addition of phosphate to the **1**·Zn^{2+} complex [17]. The time-dependence of fluorescence intensity [18] of **1**·Zn^{2+} complex was measured and the outcome of the result was compared with other anions (Fig. S10). The **1**·Zn^{2+} complex attained equilibrium immediately upon after mixing with phosphate.

3.6. Competitive anion binding of zinc complex of receptor

The competitive anion binding test was carried out with **1**·Zn^{2+} complex in the presence of 2 equiv. of one anion out of F^-, Cl^-, Br^-, I^-, NO_3^-, CN^-, AcO^-, ClO_4^- and HSO_4^- along with 2equiv of PO_4^{-3}. The fluorescence profile of **1**·Zn^{2+} with PO_4^{-3} complex was unaffected by the presence of different anions (Fig. S11). The detection limit of **1**·Zn^{2+} complex as a fluorescent sensor for the analysis of PO_4^{-3} was concluded from a plot of fluorescence intensity as a function of the concentration of the added different amounts of anion. It was found that **1**·Zn^{2+} has a detection limit of 17 μM for PO_4^{-3}. Thus **1**·Zn^{2+} can be used for the recognition and selective estimation of phosphate at industrial level. The binding of phosphate with **1**·Zn^{2+} contemplates us to study the recognition properties towards phosphorylated biomolecules. Addition of 50 μM of phosphorylated biomolecules such as ATP, ADP, AMP, NADP and NAD to 10 μM of **1**·Zn^{2+} complex in HEPES buffered DMF/H_2O (7:3, v/v) solvent system leads to quench the fluorescence emission band at 462 nm (Fig. 3C).

4. Conclusion

In conclusion, we have synthesized an "on–off" multi-responsive and selective sensor **1** as a probe to monitor the Zn^{2+} concentration through changes in fluorescence intensity based on ESIPT and charge transfer (CT) mechanism. The receptor showed high sensitivity and selectivity for Zn^{2+} detection at concentrations ranging from 0 to 100 μM with detection limit of 10 μM. The changes in the fluorescence signature of the **1**·Zn^{2+} complex in the presence of phosphate anion are significantly promising. Therefore, the Zn^{2+} complex of the receptor can be used for phosphate quantification and for the recognition of phosphorylated biomolecules through cation displacement approach. The cation displacement approach employing a metal-complex for anion recognition allows to propose new receptors for sensitive anion detection in aqueous solutions.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ica.2012.12.030.

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